ORGAN-SPECIFIC AUTOIMMUNE DISEASES INDUCED IN MICE BY ELIMINATION OF T CELL SUBSET

I. Evidence for the Active Participation of T Cells in Natural Self-tolerance; Deficit of a T Cell Subset as a Possible Cause of Autoimmune Disease

By SHIMON SAKAGUCHI, KANZO FUKUMA, KAGEMASA KURIBAYASHI, and THORU MASUDA

From the Institute for Immunology, and the Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

A number of clinical and experimental findings suggest the involvement of suppressor T cells in self-tolerance and autoimmune disease (1–3). It is, however, a matter of conjecture whether a defect in suppressor T cells plays a primary role in the pathogenesis of autoimmune disease, or only an incidental one. A definitive experiment would be one in which autoimmune disease is induced by the removal of suppressor T cells from the immune system of an otherwise normal animal, and, further, self-tolerance can be reestablished, and autoimmune disease prevented, by reconstituting such an animal with suppressor T cells.

Organ-specific autoimmune disease can be induced in rodents by treatments that deplete a subpopulation of T cells: Neonatal thymectomy of some strains of mice caused autoimmune disease in several endocrine organs (4). Autoimmune thyroiditis developed in adult rats that underwent thymectomy followed by repeated sublethal irradiation (5). Since the appearance of autoimmune disease was clearly prevented by the reconstitution of these animals with T cells from syngeneic normal animals (6, 7), it has been postulated that autoimmune disease may result from depletion of a specific T cell subpopulation responsible for checking and controlling self-reactive (i.e., autoimmune) lymphocyte clones.

In analyzing the pathogenesis of murine, organ-specific autoimmune diseases after neonatal thymectomy, we have shown (6) that neonatal thymectomy caused diminution of T cells, especially those of the Lyt-1^+, $2,3^-$ (Lyt-1) T cell subset, from peripheral lymphoid organs. Furthermore, reconstitution of thymectomized mice with Lyt-1 cells prepared from normal syngeneic mice completely inhibited disease development. These findings prompted us to examine whether direct elimination of the Lyt-1 population from a normal adult mouse could cause autoimmune disease, particularly of the organ-specific type. In this report,

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we describe our attempt to reconstitute congenitally T cell–deficient nude (*nu/nu*) mice with selected Lyt spleen cell subset(s) from euthymic (*nu/+*) heterozygotes, and studied the immunopathology of the recipient nude mice to determine whether autoimmune disease might occur.

Materials and Methods

Mice. BALB/c nude and their heterozygous (*nu/+*) littermates, originally a gift from Dr. B. Kindred, Basel Institute for Immunology, Switzerland, were obtained from the Institute of Medical Science, University of Tokyo, Japan. They were bred under specific pathogen-free conditions in the Facility of Experimental Animals at Kyoto University by mating *nu/nu* males with *nu/+* females. The age and sex of the mice used are indicated in Results. The phenotype of T cell surface antigens of BALB/c *nu/+* mice is Thy-1.2, Lyt-1.2, 2.2, 3.2.

Antisera. The antisera against Thy-1.2 and Lyt antigens were prepared by standard alloimmunization using congenic mice (8). Their titers and specificities were described previously (9). Monoclonal antibodies (mAb)[1] anti-Lyt-1.2 (γ2a,k) [anti-Lyt-1.2(m)] (10) and anti-Lyt-2.2 (γ2a,k) [anti-Lyt-2.2(m)] (11), both from ascites, were gifts from Dr. F.-W. Shen, Memorial Sloan-Kettering Cancer Center, New York, and Dr. E. Nakayama, Center for Adult Diseases, Osaka, Japan, respectively. Their titers at 50% cytotoxicity were 1:10,000 and 1:25,000, respectively, by trypan blue dye exclusion test(12).

Treatment of Cells With Antisera and Complement (C). Bulk treatment of cells was carried out as previously described (9). Briefly, 2×10^7 spleen cells in 0.2 ml of Medium 199 (Gibco Laboratories, Grand Island, NY) with 2% fetal calf serum (FCS) (Gibco Laboratories) were incubated at 4°C with conventional anti-Thy-1.2, Lyt-1.2, Lyt-2.2, or normal mouse serum (NMS) at final dilution of 1:7, with occasional vigorous shaking. After 40 min, 0.75 ml of 1:6 diluted rabbit serum, selected for high C activity and low toxicity, was added. After an additional 30 min incubation at 37°C in a waterbath, the cells were washed twice with cold medium containing 5% FCS and suspended in 0.5 ml of serum-free medium, to be injected into the mice. By this protocol, the percentage of cells eliminated in a typical experiment by each antiserum and C was as follows: NMS, 12.3%, anti-Thy-1.2, 36.1%; anti-Lyt-1.2, 35.7%; and anti-Lyt-2.2, 26.7%. mAb were used at a dilution of 1:10 of the original ascites.

Histological and Serological Examination. Organs or tissues (thyroid, parathyroid, lung, salivary gland, liver, stomach, small and large intestine, pancreas, kidneys, adrenal glands, spleen, lymph nodes, ovaries, epididymides, and testes) from individual mice were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) for histological examination. For immunofluorescence (IF) to detect autoantibodies, various normal tissues were fixed in neutral, buffered formol saline, and embedded in alcohol-miscible wax of low melting point (37°C) (polyester wax; BDH Chemicals Ltd., Poole, Dorset, United Kingdom) (13). Histological quality of tissue sections prepared by this method was better than that of frozen sections, and antigenicity tested by IF was comparable. After dewaxing with graded concentrations of ethyl alcohol and phosphate-buffered saline (PBS), the tissue sections were incubated for 30 min at 37°C with the mouse serum to be tested. After washing with PBS, they were treated with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Miles Yeda, Rehovot, Israel) diluted 1:20, incubated 30 min further at 37°C, washed, and examined with a Zeiss photomicroscope. For the detection of thyroglobulin autoantibody, the hemagglutination test using human type O red blood cells coated with mouse thyroglobulin was used (14). Circulating immune complexes were measured by conglutinin-binding assay with sera of old MRL/MpJ-*lpr/lpr* mice as positive control (15).

[1] *Abbreviations used in this paper:* FCS, fetal calf serum; IF, immunofluorescence; HE, hematoxylin and eosin; mAb, monoclonal antibody; NMS, normal mouse serum; PBS, phosphate-buffered saline.

Results

By the C-dependent cytotoxicity test, the composition of Lyt subsets among Thy-1$^+$, nylon wool–nonadherent spleen cells from 3-mo-old BALB/c *nu/+* mice was as follows: Lyt-1$^+$, 2,3,$^-$(Lyt-1) cells, 40%; Lyt-1$^+$, 2,3$^+$ (Lyt-1,2,3) cells, 50%; Lyt-1$^-$, 2,3$^+$ (Lyt-2,3) cells, negligible, if present; and Lyt-1$^-$, 2,3$^-$(Lyt$^-$) cells, 5–10% (for more about Lyt$^-$ cells, see Discussion).

Induction of Oophoritis, Gastritis, and Thyroiditis in Female nu/nu Mice. Spleen cells (4×10^7) from 10–12-wk-old female *nu/+* mice were treated with various antisera plus C, then transferred intravenously, into 6–8-wk-old female *nu/nu* mice. Recipients were killed 3 mo later, and examined histologically and serologically for the development of autoimmune disease (Table I). Neither NMS plus C, nor anti-Thy-1 plus C–treated cells caused any pathological alteration in the *nu/nu* mice. However, when the spleen cells treated with anti-Lyt-1 plus C were transferred (Table I, group *C*), ovaries, stomach, and/or thyroid gland of some of the recipients were destroyed, shown also in Figs. 1 and 2. Some mice developed disease in only one organ, others in more than one. Other organs and

TABLE I

*Induction of Autoimmune Diseases in Female nu/nu Mice by the Transfer of Spleen Lyt Subsets From Female nu/+ Mice**

Experimental group	Treatment of cells*	Number of cells treated ($\times 10^7$)	Incidence of autoimmune diseases‡		
			Oophoritis	Gastritis	Thyroiditis
A	NMS	4	0/12§	0/12	0/12
B	Anti-Thy-1.2	4	0/10	0/10	0/10
C	Anti-Lyt-1.2	4	6/12 (50.0)	3/12 (25.0)	1/12 (8.3)
D	Anti-Lyt-2.2	4	0/10	0/10	0/10
E	C + D‖	2 (each)	0/10	0/10	0/10
F	Anti-Lyt-1.2 and anti-Lyt-2.2	4	8/15 (53.3)	4/15 (26.7)	3/15 (20.0)
G	Anti-Lyt-1.2(m) and anti-Lyt-2.2(m)¶	4	6/12 (50.0)	2/12 (16.7)	1/12 (8.3)
H	Anti-Lyt-1.2, anti-Lyt-2.2, and anti-Thy-1.2	4	0/10	0/10	0/10
I	D + F**	2 (each)	0/10	0/10	0/10
J	D + F‡‡	1(D), 3(F)	0/10	0/10	0/10

* Spleen cells from 10–12-wk-old female *nu/+* mice were treated with antiserum plus C, as indicated, and transferred intravenously to 6–8-wk-old female *nu/nu* mice. Recipient *nu/nu* mice were killed 3 mo after transfer.

‡ Incidence of disease was assessed by histological examination of each organ. Autoantibodies specific for corresponding organs could be detected histologically in the mice developing disease (see also Figs. 1–4).

§ Number of mice with each disease per total number of mice in a group. Percent incidence, when disease developed, is indicated in parentheses.

‖ Mixture of two cell populations: spleen cells (2×10^7) treated with anti-Lyt-1.2 plus C and others (2×10^7) treated with anti-Lyt-2.2 plus C.

¶ Spleen cells (4×10^7) were treated like group *F*, with anti-Lyt-1.2 mAb and anti-Lyt-2.2 mAb, plus C.

** Spleen cells (2×10^7) were treated like group *D* (anti-Lyt-2.2 plus C), and 2×10^7 spleen cells like group *F* (anti-Lyt-1.2 and anti-Lyt-2.2 plus C), then combined before transfer.

‡‡ Spleen cells (10^7) were treated like group *D* (anti-Lyt-2.2 plus C) and 3×10^7 spleen cells like group *F* (anti-Lyt-1.2 and anti-Lyt-2.2 plus C), then combined before transfer.

TABLE II

Induction of Autoimmune Diseases in Male nu/nu Mice by the Transfer of Spleen Lyt Subsets From Male nu/+ Mice

Experimental group	Treatment of cells*	Incidence of autoimmune diseases‡		
		Orchitis	Gastritis	Thyroiditis
A	NMS	0/10§	0/10	0/10
B	Anti-Lyt-1.2	5/12 (41.7)	3/12 (25.0)	0/12
C	Anti-Lyt-2.2	0/10	0/10	0/10
D	Anti-Lyt-1.2 and anti-Lyt-2.2	6/15 (40.0)	3/15 (20.0)	2/15 (13.3)

* 4×10^7 spleen cells from 10–12-wk-old male *nu/+* mice were treated with the antiserum indicated, plus C, and then transferred intravenously to 6–8-wk-old male *nu/nu* mice. Recipient *nu/nu* mice were killed 3 mo later for histological and serological examination.

‡, § See footnotes to Table I.

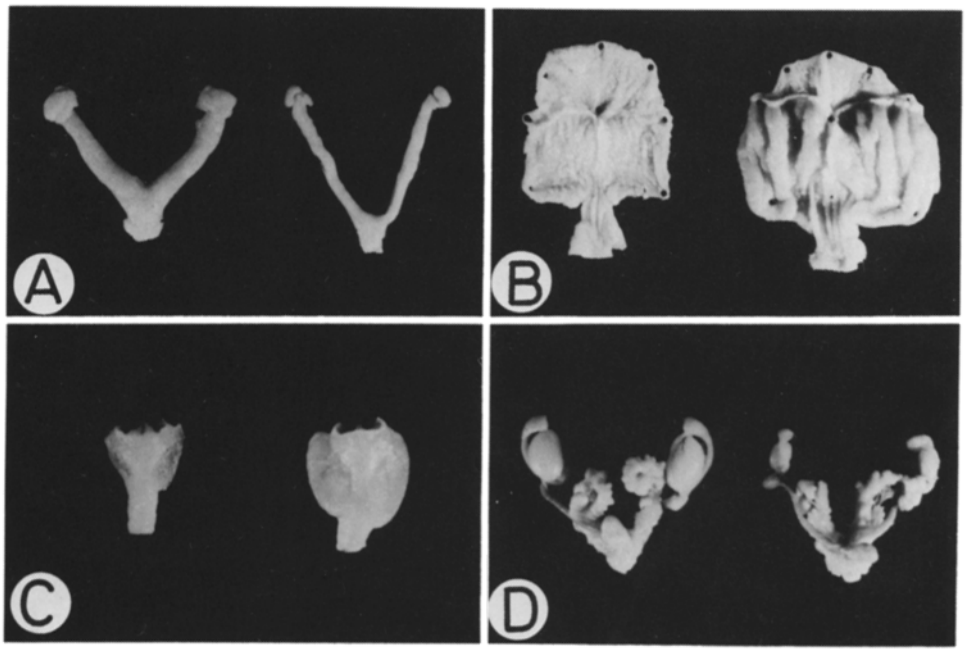


FIGURE 1. Macroscopic view of organs from *nu/nu* mice suffering autoimmune disease (Table I *F* and Table II *D*). Oophoritis, gastritis, thyroiditis, and epididymitis-orchitis (*right* side of each photograph); intact control organs from untreated *nu/nu* mice (*left*). (*A*) Ovary (× 2), (*B*) stomach (× 1.5), (*C*) thyroid (× 4), (*D*), testis (× 1).

tissues examined (see Materials and Methods) were intact, macroscopically and microscopically. We found circulating autoantibodies specific for organ components in the sera of diseased *nu/nu* mice by indirect IF (see Fig. 3). In contrast, no disease or autoantibodies appeared after transfer of *nu/+* spleen cells that were treated with anti-Lyt-2 plus C (Table I, group *D*). The reconstitution of *nu/nu* mice with a mixture of cells treated with anti-Lyt-1 plus C and anti-Lyt-2 plus C, so that Lyt-1,2,3 cells were absent, also failed to cause any disease in the recipients (Table I, group *E*).

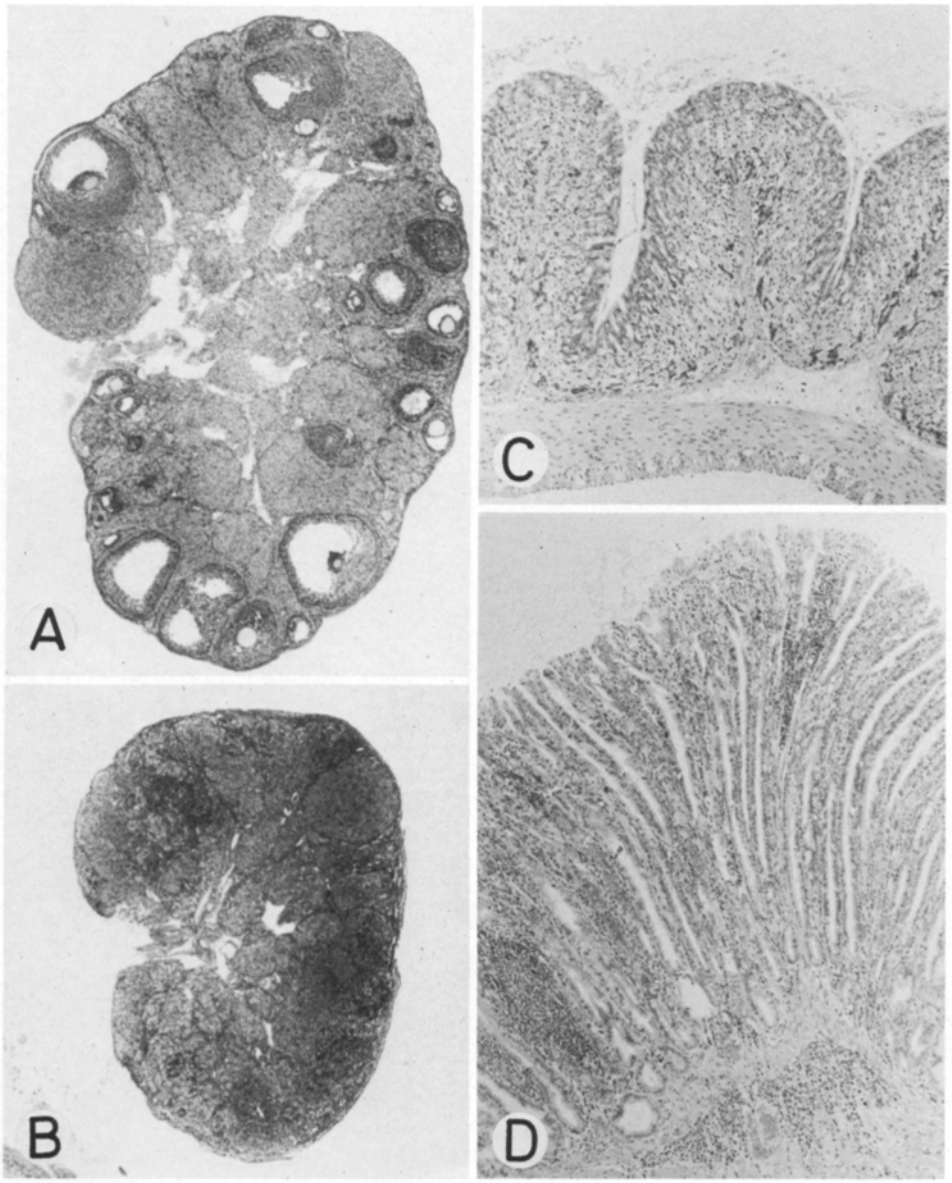
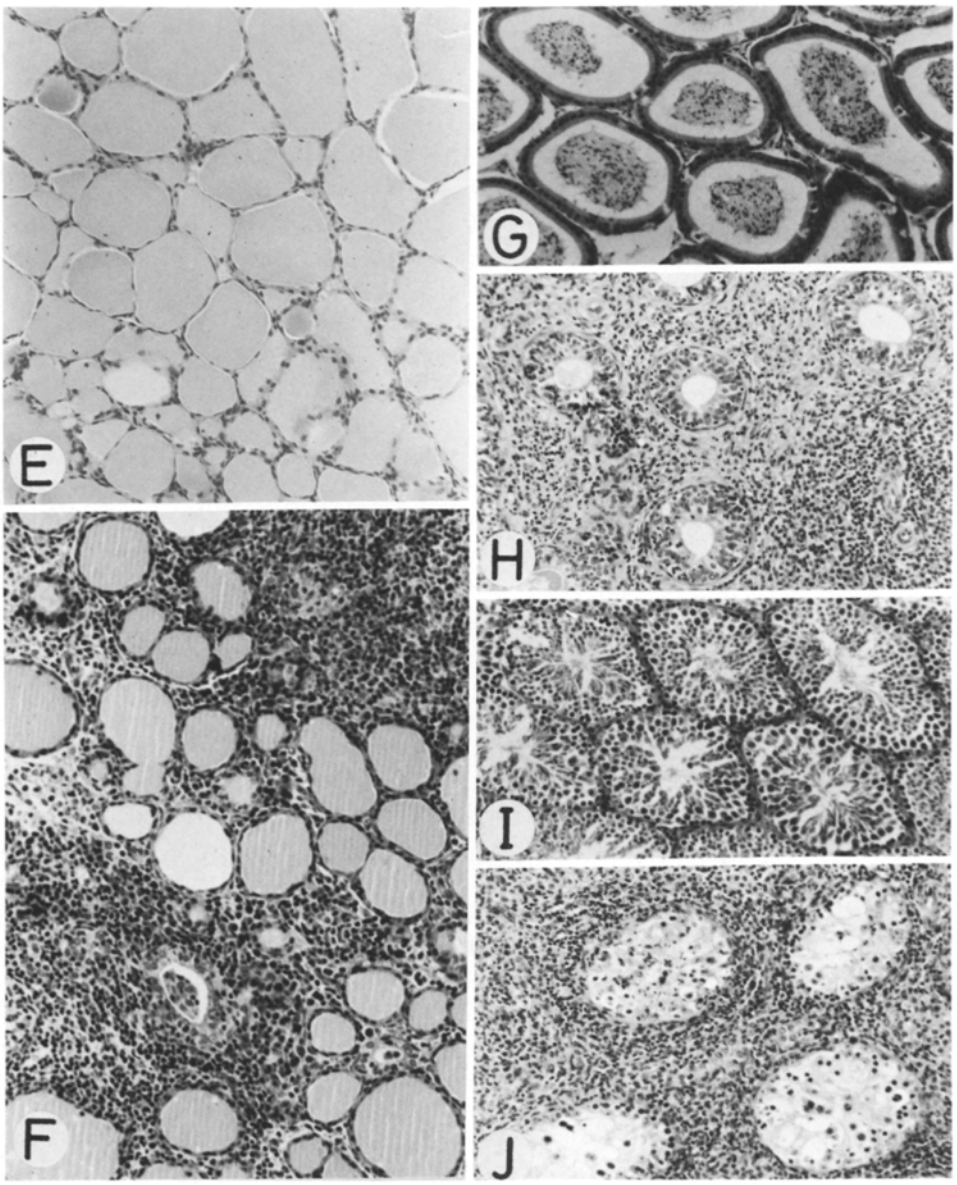


FIGURE 2. Histology of organs developing autoimmune diseases (*B, D, F, H, J*) (Table I*F* and Table II*D*); and of intact control organs from untreated *nu/nu* mice (*A, C, E, G, I*). Ripening or growing follicles, with oocytes and corpora lutea, seen in normal ovary (*A*) (× 80) are destroyed in oophoritis (*B*) (× 80) with diffuse infiltration of mononuclear cells. Gastric mucosa with normal architecture (*C*) (× 120) is damaged in gastritis (*D*) (× 120). Loss of parietal cells and compensatory hyperplasia of mucous cells result in thickening of gastric mucosa. Inflammatory cells, mainly mononuclear cells, infiltrate into the submucosa and epithelium. Acinar follicles in normal thyroid gland (*E*) (× 200) are obliterated and replaced by the interstitial cell infiltration in thyroiditis (*F*) (× 200). Ducts in normal epididymis containing mature sperm (*G*) (× 200) and seminiferous tubules with spermatogenesis in normal testis (*I*) (× 200) are damaged and replaced by massive infiltration of mononuclear cells and some polymorphonuclear cells in epididymitis (*H*) (× 200) and orchitis (*J*) (× 200). No sperm were observed, and spermatogenesis is abolished in epididymitis-orchitis. (HE stain).



After the removal of Lyt-1$^+$ cells by anti-Lyt-1 plus C treatment, Lyt-2,3 and Lyt$^-$ cells remain. To identify the cell population responsible for the induction of disease, we tried to eliminate all Lyt$^+$ cells by treating *nu*/+ spleen cells with a mixture of anti-Lyt-1 and anti-Lyt-2, plus C (Table I*F*). This population, containing Lyt$^-$ cells but not Lyt-2,3 cells, induced disease with about the same incidence as anti-Lyt-1 plus C–treated cells. Similar treatment with anti-Lyt-1 and anti-Lyt-2 mAb, plus C, induced disease with almost the same incidence as with conventional anti-Lyt sera (Table I*G*). Because treatment with mixed sera of anti-Thy-1, anti-Lyt-1, and anti-Lyt-2, plus C, completely abrogated the

disease-inducing capacity of *nu*/+ spleen cells (Table I*H*), these results, altogether, indicate that Thy-1$^+$, Lyt$^-$ cells were responsible for the induction of autoimmune disease.

As noted above, transferred cell populations that contained Lyt-1 cells did not cause disease, even if they also included Lyt$^-$ cells (Table I, *A*, *D*, and *E*). This finding was further substantiated by the transfer of Lyt-1 cells mixed with Lyt$^-$ cells in different ratios: when anti-Lyt-2 plus C–treated cells, which were presumed to contain Lyt-1 and Lyt$^-$ cells (prepared from 2×10^7 [Table I, group *I*] or 1×10^7 [Table I, group *J*] *nu*/+ spleen cells), were combined with Lyt$^-$ cells (prepared from 2×10^7 or 3×10^7 *nu*/+ spleen cells), and transferred to *nu/nu* mice, the development of all three autoimmune diseases was clearly inhibited in both groups of recipients. These results suggest that the Lyt-1 cells can suppress the ability of Lyt$^-$ cells to induce autoimmune disease.

Induction of Orchitis, Gastritis, and Thyroiditis in Male nu/nu Mice. When a spleen cell suspension (4×10^7 cells) from male *nu*/+ mice was depleted of specific Lyt subset(s) and transferred to male *nu/nu* mice, some recipients of the anti-Lyt-1 plus C–treated (Table II*B*) or anti-Lyt-1 and anti-Lyt-2 plus C–treated cells (Table II*D*) developed orchitis, gastritis, and/or thyroiditis. NMS plus C– or anti-Lyt-2 plus C–treated cells did not induce disease.

Histopathology of Each Autoimmune Disease and Circulating Autoantibodies Specific for Corresponding Organ Components. Figs. 1 and 2 show macroscopic and microscopic views, respectively, of affected organs from the *nu/nu* mice with autoimmune diseases. The ooporitic ovaries became involuted and the uterus atrophied, probably because of a deficiency of ovarian hormones (Fig. 1*A*). Destruction of gastric parietal cells, which secrete hydrochloric acid, and compensatory hyperplasia of mucous cells seem to be responsible for achlorhydria and the formation of giant rugae in the cases of gastritis (Fig. 1*B*). Goiters developed in the thyroid glands of mice with thyroiditis (Fig. 1*C*). Epididymes and testes afflicted with epididymitis and/or orchitis became involuted (Fig. 1*D*). Histologically (Fig. 2), each disease was characterized by damage and loss of specific organ components: oocytes, gastric parietal cells, thyroid follicular cells and colloid, and sperm, accompanied by the massive infiltration of inflammatory cells, mainly mononuclear cells (for further description of histology, see legend to Fig. 2).

Fig. 3 shows the presence of autoantibodies specific for corresponding organ components detected by indirect IF in the circulation of diseased *nu/nu* mice: Anti–zona pellucida antibody in oophoritis (some oophoritis-bearing mice also developed anti-ooplasm antibody), anti–parietal cell antibody in gastritis, anti-colloid antibody in thyroiditis (which included antithyroglobulin antibody, as demonstrated by the thyroglobulin-hemagglutination test described in the legend of Fig. 4), and antisperm antibody in epididymitis-orchitis.

Titers of circulating autoantibodies in individual mice receiving anti-Lyt-1 and anti-Lyt-2 plus C–treated *nu*/+ spleen cells (Table I*F*, and Table II*D*) were determined by IF (Fig. 4). In accordance with the results in Tables I and II, mice that developed histologically identified disease also had autoantibodies against corresponding organ constituents.

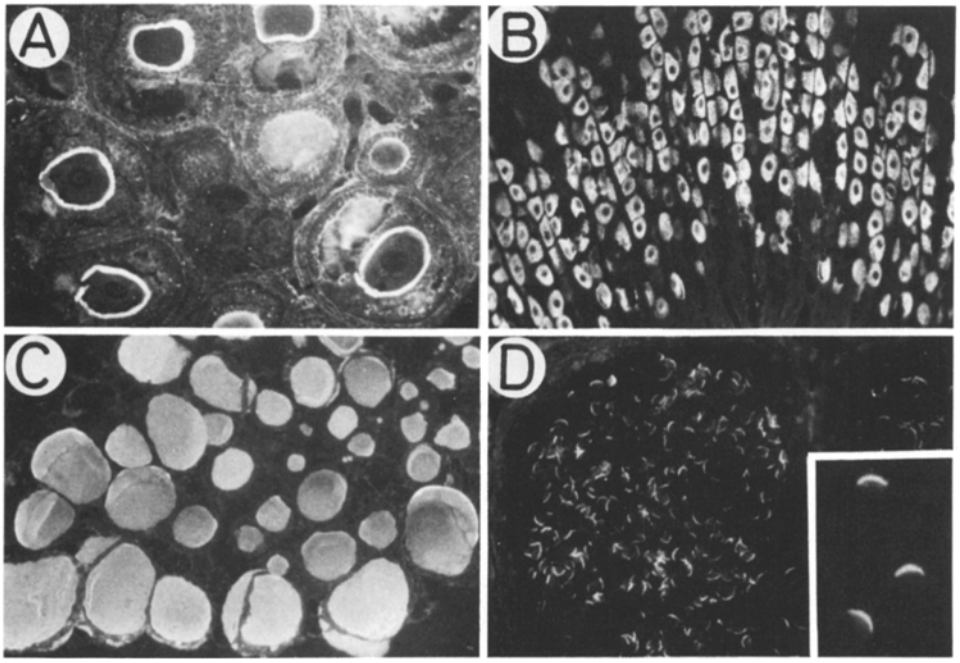


FIGURE 3. Autoantibodies specific for corresponding organs. Normal organs from *nu/+* mice are stained by the sera from *nu/nu* mice bearing each disease. (*A*) Anti-zona pellucida autoantibody in oophoritis. × 400. Ovary from 20-d *nu/+* mouse is stained. (*B*) Anti-parietal cell autoantibody in gastritis. × 400. (*C*) Anticolloid autoantibody in thyroiditis. × 200. (*D*) Antisperm autoantibody in orchitis. × 400. Staining of sperm smear is inserted. × 1600. Acrosomes of sperm are stained.

Other Autoimmune Phenomena. Under our conditions for maintaining *nu/nu* mice, ~40–50% of the untreated *nu/nu* mice ~3 mo old developed antinuclear antibodies, assessed by IF. This has also been reported by others (16). However, no significant increase in incidence or titer of antinuclear antibodies was observed in mice with organ-specific autoimmune disease. In addition, in none of the mice examined was Coombs' antibody present; circulating immune complex was not detected in the sera of the treated *nu/nu* mice, and glomerulonephritis or angitis due to immune complex desposition was not observed histologically.

Adoptive Transfer of Autoimmune Disease Into Other nu/nu Mice. Spleen cells (4 × 10^7 cells) from the disease-bearing *nu/nu* mice (Table I, *C* and *F*, and Table II, *B* and *D*) were introduced intravenously into other *nu/nu* mice of the same sex as the donors. Fig. 5 shows that the histological lesion in each disease could be specifically transferred within 60 d of the initial cell inoculation, with concomitant development of circulating specific autoantibodies. Since the treatment of donor spleen cells with anti-Thy-1 and C abrogated their capacity to transfer the disease (Table III), these results, taken together, suggest that effector T cells specific for each target organ are required for the successful transfer of these organ-specific autoimmune diseases.

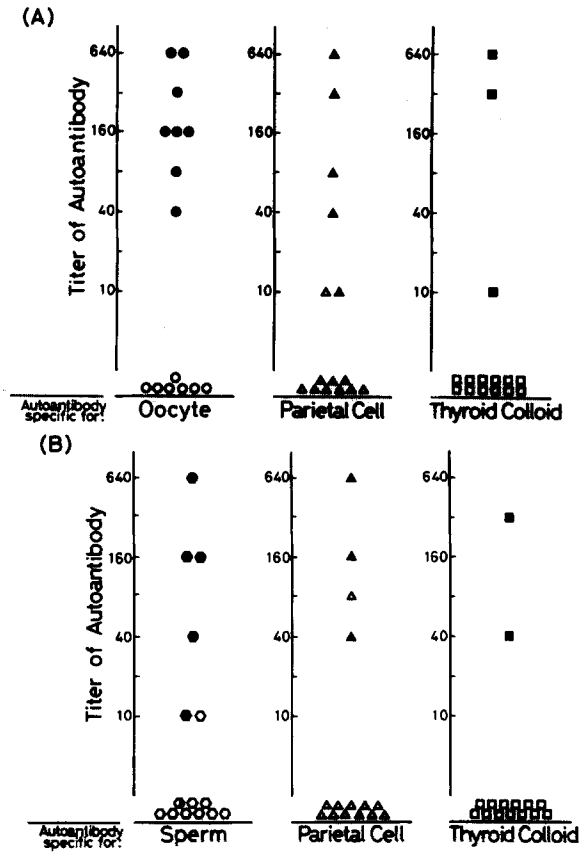


FIGURE 4. Titer of autoantibodies in the sera from female (*A*) and male (*B*) *nu/nu* mice given *nu/+* spleen cells treated with anti-Lyt-1 and anti-Lyt-2 plus C (Table I*F* and Table II*D*). (○) Histologically intact ovaries. (●) Almost all follicles and oocytes were destroyed. (△) Intact gastric mucosa. (▲) Gastritis developed macroscopically and microscopically (see also Figs. 1*B* and 2*D*). (□) Histologically intact thyroid gland. (■) Thyroiditis developed with goiter (see Figs. 1*C* and 2*F*). (◯) Intact epididymis and testis. (⬤) Both epididymitis and orchitis occurred. In the sera of thyroiditis-bearing mice, antithyroglobulin antibody was detected by the hemagglutination test: hemagglutinating titers were, for mice in *A*, ×1280, ×640, and ×320; and in *B*, ×640 and ×320, respectively.

Discussion

By dissecting splenic T cells taken from *nu/+* mice into subpopulations, based on their expression of Lyt antigens, and then transferring such Lyt subsets to *nu/nu* mice, we were able to induce autoimmune diseases in several organs, including ovary, stomach, thyroid, and testis. These organs were damaged histologically by massive infiltration of inflammatory cells, with concomitant development of autoantibodies specific for particular components in each organ: oocytes, parietal cells, thyroid colloid (including thyroglobulins), and sperm. Thy-1$^+$, Lyt$^-$ cells were shown to be responsible for inducing disease, and Lyt-1 cells, when mixed with the Lyt$^-$ cells, were shown to have suppressive activity on the development of disease.

There are some controversies concerning the expression of Lyt antigens on

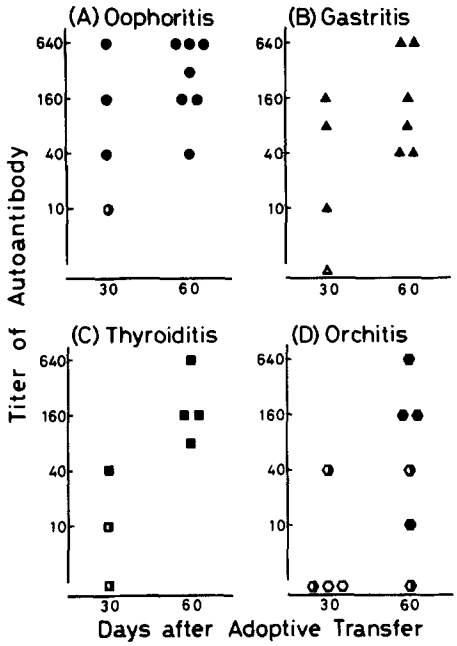


FIGURE 5. Adoptive transfer of autoimmune diseases (oophoritis, gastritis, thyroiditis, and orchitis) into other *nu/nu* mice. Spleen cells (4×10^7) from *nu/nu* mice developing each autoimmune disease (Table I, *C* and *F*, and Table II, *B* and *D*) were inoculated intravenously into 6–8-wk-old *nu/nu* mice. The recipients were killed 30 or 60 d after transfer for histological and serological examination. (○, ●, △, ▲, □, ■, ◯, ⬤) See legend to Fig. 4. (◭) Gastritis not remarkable macroscopically but histologically detectable. (◨) Thyroiditis developed histologically, but goiter was not formed yet. (◑) Epididymitis developed, but testis was histologically intact.

lymphocytes. Whether Lyt-1 antigens are expressed on all T cells, and even on B cells, when examined by such exquisite assays as cytofluorometry (17, 18) is, as yet, uncertain. The definition of Lyt^- cells in this report is an operational one, based on a C-dependent cytotoxicity assay. We do not mean to assert that Lyt^- cells are necessarily devoid of all Lyt antigens; their expression on Lyt^- cells may be too low for lysis with antibody plus C. It may be more appropriate, therefore, to designate Thy-1^+, Lyt-1^-, $2,3^-$ as Thy-1↑, Lyt-1↓, 2,3↓, as suggested by Shiku et al (19). Thy-1^+, Lyt^- cells detected by cytotoxicity or membrane IF assay have, thus far, been reported in normal spleen and in lymph nodes (20), from in vitro mixed lymphocyte reactions (21), in mitogen-stimulated T blast populations (22), from a cell line maintained by T cell growth factors (23), in lymphoadenopathy of lupus-prone MRL mice (24), and in the infiltrating cells in experimentally induced allergic thyroiditis (25). Lyt^- cells are also rich in peripheral blood lymphocytes of normal mice (Nakayama and Sakaguchi, unpublished data). Lyt^- cells, prepared by C-dependent cytotoxic depletion, seem to be a distinct T cell subset as unique as other Lyt subsets. Since the adoptive transfer experiment suggested the existence of specific effector T cells for each autoimmune disease (Fig. 5 and Table III), Lyt^- cells could be considered precursors of such effector T cells. In the cases of autoimmune oophoritis, induced by neonatal thymectomy,

TABLE III

Requirement of T Cells for Adoptive Transfer of Autoimmune Diseases

Exp.	Autoimmune disease(s) in donor *nu/nu* mouse*	Treatment of cells transferred‡	Autoimmune disease(s) induced in recipient *nu/nu* mouse§			
			Oophoritis	Gastritis	Thyroiditis	Orchitis
1	Oophoritis	NMS	+(640)‖	–	–	
		Anti-Thy-1.2	–	–	–	
2	Oophoritis, gastritis	NMS	+(320)	+(320)	–	
		Anti-Thy-1.2	–	–	–	
3	Thyroiditis	NMS	–	–	+(640)	
		Anti-Thy-1.2	–	–	–	
4	Oophoritis, thyroiditis	NMS	+(160)	–	+(640)	
		Anti-Thy-1.2	–	–	–	
5	Orchitis	NMS		–	–	+(320)
		Anti-Thy-1.2		–	–	–

* Those *nu/nu* mice with autoimmune diseases (Table I *F* and Table II *D*) were used as the spleen cell donors.

‡ Spleen cells (4×10^7) from disease-bearing *nu/nu* mice were treated. ~15% of the donor spleen cells were Thy-1^+ by cytotoxicity test.

§ Donor *nu/nu* mice and recipient *nu/nu* mice were of the same sex. Recipient *nu/nu* mice aged 6–8 wk were inoculated with spleen cells intravenously, and killed 60 d later.

‖ Autoimmune disease developed histologically (+), as shown in Figs. 1 and 2. Organ histologically intact (–). Titer of autoantibodies specific for the corresponding organ components was assessed by IF, as shown in Fig. 5. Values are given in parentheses for titers ≥10.

and experimental allergic thyroiditis, the autoimmune diseases can be adoptively transferred to syngeneic mice or nude mice by Lyt-1 cells alone (9), or by a Lyt-1 T cell line maintained in vitro by exogenous growth factors (26). It is conceivable, therefore, that the Lyt$^-$ (Lyt-1↓, 2,3↓) population might give rise to Lyt-1 effector T cells. Since nude mice are known to have a small number of Thy-1^+ cells (27), it is also plausible that the transferred *nu/+* Lyt$^-$ cells might interact with *nu/nu* Thy-1^+ cells and induce them to differentiate into effector cells. These possibilities are now under investigation.

Lyt-1 cells have been shown to have suppressive activity on cellular and humoral immunity in an antigen-specific or -nonspecific manner (28–30). Recent investigations (31, 32) of T cell regulatory mechanisms in various immune responses have revealed suppressor circuits or cascades. These circuits involve interactions among Lyt subsets, via suppressive factors and/or antiidiotypic antibodies. The role of Lyt-1 cells as inducers (31) or first-order suppressors has received special emphasis (32). In our experiments, Lyt-1 cells were sufficient to suppress the development of disease, apparently by acting directly upon Lyt$^-$ cells. Further investigation of Lyt-1 cells and their mode of action in actively regulating self-reactive clones is needed, especially to determine whether they are specific for each organ-antigen, and, if so, when and how they are sensitized to self-antigens to acquire regulatory activity. Other Lyt subsets and their

interactions with Lyt-1 cells might also be involved if Lyt^- cells can differentiate into other (Lyt^+) subsets.

In nude mice or mice depleted of T cells (by thymectomy, irradiation, and reconstitution with bone marrow cells), no organ-specific autoimmune disease develops spontaneously under control conditions. Nor can it be induced by exogenous immunization with self-antigen such as thyroglobulin and adjuvant (33). In the present experiment, *nu/+* spleen cell suspensions freed of a particular T cell subset (i.e., Lyt-1^+ cells) could induce autoimmune disease in nude mice, but those depleted of all T cells could not. Together with the necessity for T cells in adoptive transfer, these findings suggest that T cells may be required, not only for the maintenance of self-tolerance, but also for inducing autoimmunity, leading to eventual destruction of self-tissues. Without constant help from the effector T cells, B cells seem to be incapable of forming autoantibodies quantitatively or qualitatively sufficient to cause tissue damage, even if they may have the repertoire for self-constituents. This dependency has been demonstrated for human peripheral B cells binding thyroglobulin (34); the development of such self-reactive T cells, including helper T cells for autoantibody formation and/or T cells mediating cellular autoimmune reactions (9), may, in turn, be checked and controlled by another T cell subset(s), in a healthy condition.

With respect to the spectrum of organs affected, and immunopathological features of individual diseases and their chronicity, the autoimmune diseases induced in the nude mice are similar to human organ-specific autoimmune diseases: Hashimoto's thyroiditis, atrophic gastritis with pernicious anemia, premature ovarian failure with antioocyte autoantibody (35), and male infertility with antisperm autoantibody. In the human, one organ-specific autoimmune disease is frequently associated with another (36). For organ-specific autoimmune disease to be complicated with a non–organ-specific one, such as systemic lupus erythematosus, however, is rare (37). It is noteworthy, therefore, that even when the nude mice developed autoimmune disease on more than one organ, they did not develop non–organ-specific autoimmunity or immune complex disease, as seen in the lupus-prone NZB or MRL strains of mice (38), or mice with chronic graft-vs.-host disease (39). Conversely, in these models of systemic autoimmunity, few organ-specific autoimmune diseases, if any, have been reported (38, 39). This suggests that the pathogenic mechanisms of these two groups of autoimmunities may be different.

Whether there might be some unifying element among the current theories of immunological tolerance to both self- and non–self-antigens is still in question, i.e., clonal deletion (40), T and/or B cell inactivation (41–43), or active regulation by suppressor T cells (44). Accordingly, it is also a matter of debate whether the pathogenic mechanism of autoimmune disease can be explained by any single concept. Forbidden clones (40), T cell bypass mechanisms (2, 43), or defects in suppressor T cells (45) need to be thoroughly considered. Our present study strongly suggests that organ-specific autoimmune diseases can be produced by a deficit or defect in a particular T cell subset(s) that appears to have a suppressive effect on self-reactive lymphocytes. Active cellular interactions among T cell subsets, the suppressor subset being dominant in the healthy state, is presumably responsible for maintaining physiological self-tolerance to some self-constituents.

Environmental agents, infectious or toxic, may exist which selectively affect the suppressor subset to cause organ-specific autoimmune diseases.

Summary

Organ-specific autoimmune diseases such as oophoritis, gastritis, thyroiditis, and orchitis were induced in female or male nude (*nu/nu*) mice by the transfer of *nu/+* spleen cells from which particular Lyt T cell subset(s) had been removed: *nu/+* spleen cells treated with anti-Lyt-1 plus complement (C) caused disease in recipient nude mice; anti-Lyt-2 plus C–treated spleen cells, in contrast, did not. The cells responsible for disease induction are believed to be Thy-1$^+$, Lyt-1$^-$, 2,3$^-$ (Thy-1↑, Lyt-1↓, 2,3↓), since spleen cells treated with mixed antisera, including anti-Lyt-1 and anti-Lyt-2, plus C, could induce the disease with almost the same incidence as anti-Lyt-1 plus C–treated cells (oophoritis 50%, gastritis 25%, thyroiditis 10–20%, and orchitis 40%). Cells treated with mixed antisera of anti-Thy-1, anti-Lyt-1, and anti-Lyt-2, plus C, could not induce autoimmune disease.

Each induced autoimmune disease could be adoptively transferred to other nude mice via spleen cells, with resulting histological lesion of corresponding organs and development of specific circulating autoantibodies. Since anti-Thy-1 plus C treatment of donor spleen cells abrogated the capacity to transfer the disease, we conclude that T cells are required as effector cells, and that these may develop from Lyt-1$^-$, 2,3$^-$ cells.

Lyt-1$^+$, 2,3$^-$ cells were demonstrated to have suppressive activity upon the development of the diseases; induction of autoimmunity was completely inhibited by the cotransfer of Lyt-1$^+$, 2,3$^-$ cells with Lyt-1$^-$, 2,3$^-$ cells. When anti-Lyt-2 plus C–treated cells (i.e., Lyt-1$^+$, 2,3$^-$ and Lyt-1$^-$, 2,3$^-$ cells) were mixed with anti-Lyt-1 and anti-Lyt-2 plus C–treated cells (i.e., Lyt-1$^-$, 2,3$^-$ cells) in various ratios, then transferred to nude mice, the development of each autoimmune disease was clearly inhibited, even by small doses of Lyt-1$^+$, 2,3$^-$ cells.

The autoimmune disease we were able to induce was quite similar to human organ-specific autoimmune disease in terms of the spectrum of organs involved, histopathological features, and the development of autoantibodies to corresponding organ components (oocytes, parietal cells, thyroid colloid, including thyroglobulin, and sperm). The results offer direct evidence for the active participation of T cells in natural self-tolerance to certain self-constituents, and suggest that a deficit or defect in certain T cell subset(s) is a possible cause of organ-specific autoimmune disease in humans.

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